

**ICCVAM Test Method Evaluation Report: Recommendation to
Discontinue Use of the Low Volume Eye Test for Ocular Safety
Testing**

**Interagency Coordinating Committee on the
Validation of Alternative Methods**

**National Toxicology Program Interagency Center for the
Evaluation of Alternative Toxicological Methods**

**National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
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List of Abbreviations and Acronyms

°C	Degrees centigrade
AHT	Animal health technologist
BRD	Background review document
CPSC	U.S. Consumer Product Safety Commission
CV	Coefficient of variation
ECVAM	European Centre for the Validation of Alternative Methods
EPA	U.S. Environmental Protection Agency
ESAC	European Centre for the Validation of Alternative Methods Scientific Advisory Committee
EU	European Union
FDA	U.S. Food and Drug Administration
FR	<i>Federal Register</i>
g	Gram
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
GLP	Good Laboratory Practice
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ILS	Integrated Laboratory Systems, Inc.
IS	Irritation Score
JaCVAM	Japanese Center for the Validation of Alternative Methods
kg	Kilogram
LVET	Low volume eye test
MAS	Maximum average score
MeSH	Medical Subject Headings
mg	Milligram
mL	Milliliter
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NSAID	Nonsteroidal anti-inflammatory drug
NTP	U.S. National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OTWG	ICCVAM Ocular Toxicity Working Group
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SC	Subcutaneous
SRD	Summary review document
TG	Test guideline
TSA	Test substance administration

UN	United Nations
U.S.	United States

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Preface

Eye injury is a leading cause of visual impairment in the United States with 40,000 to 50,000 new cases of impaired vision reported each year.¹ Many eye injuries occur due to contact with workplace or household products or chemicals. Accidents involving common household products (e.g., oven cleaner and bleach) cause about 125,000 eye injuries each year.² These products often result in chemical burns and emergency room visits.³ Each day about 2,000 U.S. workers have a job-related eye injury that requires medical treatment. Although the majority of these eye injuries result from mechanical sources, chemical burns from industrial chemicals or cleaning products are common.⁴

To prevent eye injuries, regulatory agencies require testing to determine if chemicals and products may cause eye damage. This testing information is used to classify the ocular hazard and determine appropriate labeling to warn consumers and workers of the potential hazard. Appropriate labeling tells users how to avoid exposure that could damage the eye and what emergency procedures should be followed if there is accidental exposure. Nearly all ocular safety testing has been conducted using the Draize rabbit eye test, although *in vitro* methods can now be used to identify whether substances cause severe irritation or permanent eye damage. The Draize rabbit eye test (Draize et al. 1944) involves instillation of 0.1 mL of the test substance into the conjunctival sac of one eye. The other eye serves as the untreated control. The eye is examined at least daily for up to 21 days. The presence and severity of any injuries to the cornea, conjunctiva, and the iris (tissues inside the eye) are scored and the duration that the injuries persist is recorded.

More recently, Griffith et al. (1980) developed the low volume eye test (LVET) with the intention that it would more accurately reflect the human response, since the traditional Draize rabbit eye test was considered to consistently overpredict the human ocular hazard potential. The LVET differs from the Draize rabbit eye test in that only 10% of the volume used in the Draize is applied to the eye (10 μ L vs. 100 μ L), and the test substance is applied directly on the center of the cornea instead of in the conjunctival sac.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently reviewed the validity of the LVET as a replacement for the Draize rabbit eye test. This was necessary because LVET data were used to support the validity of a proposed non-animal *in vitro* testing strategy for antimicrobial cleaning products. As a part of this evaluation, ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) requested the submission of data and information on substances tested in rabbits using the LVET protocol (73 FR 18535).⁵

ICCVAM carefully compiled and assessed all available data and arranged an independent scientific peer review. ICCVAM and the Ocular Toxicity Working Group (OTWG) solicited and considered public comments and stakeholder involvement throughout the evaluation process. As part of their ongoing collaboration with ICCVAM, scientists from the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM) served as liaisons to the OTWG. ICCVAM, NICEATM, and the OTWG prepared a draft summary review document (SRD) describing the validation status of the LVET, including its reliability and accuracy, and draft test method recommendations for its usefulness and limitations. ICCVAM released this document to the public for comment on March 31, 2009. ICCVAM also

¹ Available at http://www.preventblindness.org/resources/factsheets/Eye_Injuries_FS93.pdf

² Available at <http://www.geteyesmart.org/eyesmart/injuries/home.cfm>

³ From the CPSC NEISS Database, 2007

⁴ Available at <http://www.cdc.gov/niosh/topics/eye/>

⁵ Available at <http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-E8-6969.pdf>

announced a meeting of the independent international scientific peer review panel (Panel) (74 FR 14556).⁶

The Panel met in public session on May 19–21, 2009, to review the ICCVAM draft SRD for completeness and accuracy. The Panel then evaluated (1) the extent to which the draft SRD addressed established validation and acceptance criteria and (2) the extent to which the draft SRD supported ICCVAM's draft test method recommendations. Before concluding their deliberations, the Panel considered written comments and comments made at the meeting by public stakeholders.

ICCVAM provided the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) with the LVET draft SRD and draft test method recommendations, a summary of the conclusions and recommendations from the Panel meeting, and all public comments for discussion at their meeting on June 25–26, 2009, where public stakeholders were given another opportunity to comment. A detailed timeline of the evaluation is included with this report.

ICCVAM solicited and considered public comments and stakeholder involvement throughout the test method evaluation process. ICCVAM considered the SACATM comments, the conclusions of the Panel, and all public comments before finalizing the ICCVAM test method recommendations. The recommendations and the SRD, which is provided as an appendix to this report, are incorporated in this ICCVAM test method evaluation report. As required by the ICCVAM Authorization Act, ICCVAM will forward its recommendations to U.S. Federal agencies for consideration. Federal agencies must respond to ICCVAM within 180 days after receiving the ICCVAM test method recommendations. ICCVAM recommendations are available to the public on the NICEATM–ICCVAM website.⁷ Agency responses will also be made available on the website as they are received.

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⁶ Available at <http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/E9-7220.pdf>

⁷ Available at <http://iccvam.niehs.nih.gov/methods/ocutox/AMCP.htm>

Executive Summary

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently evaluated the validation status of the *in vivo* low volume eye test (LVET). This test method evaluation report provides ICCVAM's recommendations on the usefulness and limitations of the LVET as an alternative to the Draize rabbit eye test (Draize et al. 1944) for assessing substances' ocular irritation potential.

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, ICCVAM, and its Ocular Toxicity Working Group prepared a summary review document (SRD). The SRD, which summarizes the current validation status of the LVET, is based on published studies and forms the basis for draft ICCVAM test method recommendations. The draft SRD and ICCVAM recommendations were provided to an independent international scientific peer review panel (Panel) and to the public for comment. A detailed timeline of the ICCVAM evaluation process is appended to this report.

The Panel met in public session on May 19–21, 2009, to discuss its peer review of the ICCVAM draft SRD. The Panel members discussed how well the information contained in the draft SRD supported ICCVAM's draft test method recommendations. In finalizing this test method evaluation report and the SRD, which is included as an appendix, ICCVAM considered (1) the conclusions and recommendations of the Panel, (2) comments from ICCVAM's Scientific Advisory Committee on Alternative Toxicological Methods, and (3) public comments.

Specific ICCVAM Test Method Recommendations

Test Method Usefulness and Limitations

ICCVAM does not consider the LVET a valid replacement for the Draize rabbit eye test. Accordingly, ICCVAM does not recommend the LVET for prospective ocular safety testing. If animals must be used for ocular safety testing, ICCVAM recommends using the modified Draize rabbit eye test protocol that incorporates the recommended topical anesthetics, systemic analgesics, and humane endpoints. However, ICCVAM concluded that retrospective LVET data can be used in a weight-of-evidence approach to classify ocular hazards provided that the validity of each type of evidence used for such assessments is adequately characterized.⁸

ICCVAM recommends using Draize data to select reference chemicals for all future validation studies of new, revised, and alternative test methods for ocular safety testing. Priority should be given to chemicals for which there are both Draize data and human data (e.g., from accidental exposures or standardized ethical human studies).

Test Method Protocol

As indicated above, ICCVAM does not recommend any future testing using the LVET and therefore does not recommend a test method protocol.

Future Studies

ICCVAM recommends that additional requests be made for available historical data that participating companies may have on the LVET (e.g., in-house or external studies they have supported, or research and testing studies). Where such data are available, efforts should be made to determine (1) which could be used in a weight-of-evidence approach and (2) how they might be considered.

⁸ The ECVAM Scientific Advisory Committee (ESAC) does not consider the LVET a valid replacement for the Draize rabbit eye test. ESAC also concludes that retrospective LVET data can be used in a weight-of-evidence approach to classify ocular hazards (ESAC 2009; **Appendix D**).

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